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SCREENING OF F1 HYBRIDS AND THEIR PARENTS FOR RESISTANCE TO MULTIPLE VIRUSES IN CHILLI (*CAPSICUM ANNUUM* L.)

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ABSTRACT

Among the viruses infecting chili, potato virus Y (PVY), chili veinal mottle virus (CVMV), tobacco etch virus (TEV), pepper vein banding virus (PVBV), pepper veinal mottle virus (PVMV) belonging to potyvirus group and cucumber mosaic virus (CMV) belonging to cucumovirus are economically the most important virus transmitted mechanically, and by aphids in a non persistent manner. In the present investigation, totally 16 parents and their 55 F1 hybrids were screened for four major viruses infecting chili viz., PVY, CVMV, PVBV and CMV. Seedlings were inoculated by mechanical sap inoculation method. Young leaves were observed for symptoms after 2 weeks of inoculation. In parental lines- PMR-57, RHRC-50-1, PMR-76, PMR-37, MI-2, AR-27, AR-28, AR-52, Perennial, Punjab Lal, Punjab Guchedhar, Pant C-1 and Tiwari. Crosses PMR-57 x Perennial, PMR-57 x Tiwari, RHRC-50-1 x Perennial, RHRC-50-1 x Punjab Guchedhar, PMR-69 x Perennial, PMR-69 x Perennial, PMR-69 x Punjab Guchedhar, PMR-69 x Tiwari and AR-28 x Perennial were expressed resistance to at least three viruses tested, and sixteen crosses expressed resistance to at least two viruses. Cross PMR-57 x Punjab Guchedhar expressed resistance to all the four viruses studied.

KEYWORDS: Chilli, Capsicum Annuum, Virus Resistance, Potyviruses & Cucumber Mosaic Virus

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Original Article

INTRODUCTION

About 45 viruses are known to be infected peppers (Capsicum *spp*), of these, 21 viruses are known to be infected in India. Among these, potato virus Y (PVY), chili veinal mottle virus (CVMV), tobacco etch virus (TEV), pepper vein banding virus (PVBV), pepper veinal mottle virus (PVMV) belonging to potyvirus group and cucumber mosaic virus (CMV) belonging to cucumovirus are economically the most important virus transmitted mechanically and by aphids in a non persistent manner (Green and Kim, 1991; Siriwong *et al*., 1995; Ravi *et al*., 1997). Potyviruses and tobamoviruses are destructive in tropical countries (Green and Kim, 1991; Krishna Reddy and Singh, 1995).

The crop losses in Chili vary with variety, virus and region (Koennings and McClure, 1981). Yield loss due to CMV was found to be more than 60 per cent (Joshi and Dubey, 1973; Florini and Zitter, 1987), and 50 per cent due to CVMV (Ong et. Al., 1980), 40 to 100 per cent due to TMV infection and 70 percent due to TEV infection (Villalon, 1981). The damage caused by viruses depends upon the strains involved, the host cultivars grown and whether the viruses occur singly or in mixed infections.

In India, a few varieties like Pant C-1, Tiwari, Punjab Lal and some other varieties are developed for resistance to viruses in chilies is a common feature in nature, there is a strong need to develop multiple virus

resistant variety/hybrids with desirable fruit type.

The host-virus research has been paying ever increasing attention to seek out plants, resistant to viruses and detecting the occurrence of virus resistance genes. The importance lies in the fact that the chemotherapy and physiotherapy procedures used *in vivo* against viruses are inapplicable in practice, the conventional plant protection methods are of poor efficiency and undesirable from the point of environmental protection. These facts suggest that the control of the virus pathogens of Chile is restricted exclusively to breeding for virus resistant varieties/hybrids.

MATERIALS AND METHODS

Screening of Parents and F1 Hybrids for Virus Resistance

Totally 16 parents and 55 F1 hybrids were screened for four major viruses infecting chili viz., Potato virus Y (PVY), Pepper vein banding virus (PVBV), chili veinal mottle virus (CVMV) and cucumber mosaic virus (CMV) in insect proof screen house.

The parents, which were screened in October 1999 for all four viruses, were again screened. The seeds of all 16 parents and 11 hybrids were sown in seed pans at the first stage of screening. After 10 days, another 11 hybrids were sown; similarly all the hybrids were sown at 10 days interval for convenience. Twenty days old seedlings of each parent and its corresponding hybrids were transplanted to pot with 5 seedlings.

Stage of Inoculation

Seedlings in 3-4 leaf stage were inoculated.

Method of Transmission of Virus

Mechanical sap inoculation: PVY, PVBV, CVMV and CMV, all these viruses can be transmitted mechanically through sap inoculation. Preparation of inoculums of PVY, PVBV, CVMV and CMV: Young leaves showing mosaic symptoms on *Nicotiana glutinosa* were used as a standard source of inoculums of PVY. Young leaves showing mosaic, mottling, vein clearing symptoms on *Nicotiana glutinosa* was used as a standard source of inoculums of PVBV and young leaves showing symptoms of mosaic, mottling on *Datura metal* as standard inoculums of CVMV. Young leaves showing systemic mosaic, and local lesion of Cucumis sativus were used as a standard source of inoculums of CMV.

The inoculums were prepared by macerating the leaves in a sterilized pestle and mortar, in the presence of 0.05M phosphate buffer (pH 7.0) at 1:3 (w/v) weights by volume of leaf tissue. After maceration, the pulp was squeezed through sterilized absorbent cotton. The extract was used as standard inoculums for inoculation.

Colette (600 mesh size) was added at the rate of 0.025 g per ml of the standard inoculums. Inoculation was done by dipping the forefinger in the inoculums and simmering gently on the upper surface of the leaves. Then, the inoculated leaves were washed immediately within 5 to 10 minutes, with jet of water from a squeeze bottle, and the plants were kept under observation, till symptoms appeared and advanced, nearly up to 50 days.

RESULTS AND DISCUSSIONS

Reaction of Parents and F1 Hybrids Developed to Different Viruses Tested

Pepper Vein Banding Virus Resistance

Four testers Perennial, Punjab Cuchedhar, Pant C-1 and Tiwari were found to be susceptible under screen house

condition, whereas, Punjab Lal was found to be tolerant. However, all the testers expressed resistance in the field condition. This indicates the field resistance or vector non-preference. In the present investigation, these lines were not screened against aphid vectors. Out of eleven lines tested, P2, P9 and P10 were found to be tolerant, both under screen house and field condition. The line P7 was found susceptible in the screen house condition and tolerant under field conditions. The tolerance under field conditions may be attributed to natural escape resistance to vectors.

The majority of the crosses expressed susceptibility under screen house and tolerance or resistance under field conditions, which is similar to the house of testers, indicating dominant factors may be responsible. This resistance expression in the field, both in lines and hybrids clearly indicates, resistance is to the insect vector rather than a virus.

Simmonds and Harrison (1956) reported that resistance to PVBV a strain of PVY was due to two unlinked recessive loci. However, this virus is reported as a strain of PVY in 1956 by Simmonds. The present PVBV was isolated from Bangalore and found to be a different virus belonging to potyvirus group. This virus has less than 80 per cent coat protein homology with PVY strains, and therefore, it is a different virus (Joseph and Savitri, 1999). This may be a reason for the resistance behavior to be different from Simmonds and Harrison (1956).

Potato Virus Y Resistance

All testers used were found to be resistant to PVY, both under screen house and field condition.

Perennial, Punjab Lal and Pant c-1 were earlier reported as resistant to PVY, which are similar to the present investigation (Singh, 1992; Singh and Cheema, 1989; George *et al*., 1992; Konai and Nariani, 1980). All the lines were found resistant or tolerant, both under screen house and field condition. Out of 55 crosses developed, the majority of the crosses expressed resistance both under screen house and field, whereas, few expressed tolerance. This may be due to the resistance of both the parents. In the course of genetic studies on resistance to PVY, Cook and Anderson (1960), Sharma *et al*., (1989), Singh and Chenulu (1985) pointed out that, the course of inheritance of resistance was recessive, while the susceptibility of the pepper strains was a dominant hereditary feature. A dominant allele from *Capsicum annuum* line Crillo de Morlos 334, *Pvr* 4 has been conferred dominant resistance to different prototypes (0, 1 and 1,2 of PVY) (Dogimont *et al.*, 1996). Safaris and Marco (1980) reported partial dominance to PVY in Capsicum. They indicated that the heterozygous plants are more vigorous and contain lower virus titer than the homozygous plants.

Chili Veinal Mottle Virus Resistance

Testers Perennial, Punjab Lal and Pant C1 have shown resistance, both under screen house and field condition, whereas, Punjab Guchedhar and Pant C-1 showed tolerance in screen house condition and resistance in field condition. Perennial, Punjab Lal and Pant C-1 and Tiwari were already reported to be resistant to CVMV (Chew and Ong, 1990) Anon, 1993), which is similar to the present result, however, Tiwari was found tolerant in the present investigation.

Susceptible line AR-27, when crossed with resistant testers Perennial, Punjab Lal and Pant C-1, the resulting hybrids were found resistant/tolerant, indicating dominant factors involved in CVMV resistance. Quarantine and Palloix (1996) made similar observations earlier.

Lines PMR-76 and AR-52, which were tolerant to CVMV when crossed with resistant testers, the resulting hybrids were found to be susceptible under screen house conditions and resistant under natural conditions. The susceptibility of F1 generation from tolerant and resistant parents may be due to heterozygous condition. This indicates the

effects of single allele is insufficient even when one allele from each parent is present, resulting in systemic infection (Subramanya, 1982).

Cucumber Mosaic Virus Resistance

In the present investigation, Perennial found to be susceptible to CMV under screen house conditions, producing mild symptoms and resistant under field condition. This resistance in the field may be due to non-preference of the vector, however, screening with vector was not done in the present investigation. The susceptibility may also be explained by strain variation in CMV (Pinaki Acharya, 1999; Grube *et al.,* 2000). The inconsistency of resistance in Perennial from immune to susceptibility was also reported earlier, by several workers (Singh and Thakur, 1980; Pochard *et al.,* 1983; Singh, 1992).

In the present investigation, Punjab Lal was found to be resistant to CMV under field condition and tolerant under screen house condition. Field resistance to CMV was reported by Singh and Singh (1995), Singh and Cheema (1989), Singh and Kaur (1986), Singh (1992) and Bansal *et al.* (1992). However, they did not report screening under artificial conditions. Pant C-1 and Tiwari were reported to be having field resistance or tolerance to CMV (Singh, 1992; George *et al*., 1992; Singh and Kaur, 1986). In the present investigation also, Pant C-1 showed tolerance, but Tiwari showed susceptibility to CMV both under field and screen house condition.

The resistant lines PMR-57, RHRC-50-1 and PMR-69 when crossed with field resistant testers, Perennial and Punjab Lal and majority of the resulting hybrids have shown resistance, and a few have shown to CMV indicating dominant factors for resistance.

The best characterized resistance source, Perennial has been described as having monogenic recessive, partially dominant or polygenic recessive resistance, by many workers (Lapidot *et al*., 1997; Pochard and Dobbeze, 1989; Singh and Thakur, 1997; Grube *et al.* 2000). Similar observations were also seen in the present investigation in various cross combination *viz*., PMR-69 x Perennial, PMR-37 x Punjab Guchedhar, MI-2 x Tiwari and AR-52 x Punjab Lal.

Multiple Virus Resistance

Co-segregation of resistance to three potyviruses including pepper mottle virus (PepMoV), tobacco etch virus (TEV) and potato virus Y (PVY) has been observed in pepper (*Capsicum annuum*) for many years (Cook, 1960). A number of *Capsicum* genotypes are resistant to various ranges of potyvirus isolated, are currently considered to be strains of PepMoV, TEV and PVY, and a number of resistant genes have been identified, to which, resistance to more than one virus was attributed (Cook and Anderson, 1960; Dogimont *et al*., 1996; Kuhn *et al.*, 1989; Kyle and Palloix, 1997; Sowel and Demiski, 1997; Zitter, 1972). In the present investigation also, resistance to poyvirus and cucumovirus was observed in parental lines- PMR-57, RHRC-50-1, PMR-76, PMR-37, MI-2, AR-27, AR-28, AR-52, Perennial, Punjab Lal, Punjab Guchedhar, Pant C-1 and Tiwari. Crosses PMR-57 x Perennial, PMR-57 x Tiwari, RHRC-50-1 x Perennial, RHRC-50-1 x Punjab Guchedhar, PMR-69 x Perennial, PMR-69 x Perennial, PMR-69 x Punjab Guchedhar, PMR-69 x Tiwari and AR-28 x. Perennial were expressed resistance to at least three viruses tested and sixteen crosses expressed resistance to at least two viruses. Cross PMR-57 x Punjab Guchedhar expressed resistance to all the four viruses studied.

CONCLUSIONS

There is urgent need to develop multiple virus resistant hybrids in chilli. In farmers' fields, multiple virus diseases occur at a time. In the present investigation, we made an attempt to identify the sources of resistance to major viral diseases, by screening sixteen parental lines. Parental lines were crossed in a line x tester mating design to develop 55 F1 hybrids. All the parents and F1 hybrids were screened for resistance to PVY, CVMV, PVBV and CMV viruses. We could identify PMR-57, RHRC-50-1, PMR-76, PMR-37, MI-2, AR-27, AR-28, AR-52, Perennial, Punjab Lal, Punjab Guchedhar, Pant C-1 and Tiwari as resistant lines to potyviruses and cucumber mosaic virus. Crosses PMR-57 x Perennial, PMR-57 x Tiwari, RHRC-50-1 x Perennial, RHRC-50-1 x Punjab Guchedhar, PMR-69 x Perennial, PMR-69 x Perennial, PMR-69 x Punjab Guchedhar, PMR-69 x Tiwari and AR-28 x Perennial expressed resistance to at least three viruses tested and sixteen crosses expressed resistance to at least two viruses. Cross PMR-57 x Punjab Guchedhar found resistant to all the four viruses. Further investigations are necessary to find the nature of gene action.

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APPENDICES

Table 1: Reaction of Hybrids to Pepper Vein Banding Virus (PVBV) under Screen House and Field Condition in Chilli

	Perennial (S,R)		Punjab Lal (T,R)		Punjab Guchedhar (S,R)		Pant C-1 (S,R)		Tiwari (S,R)	
	SH	F	SH	F	SH	F	SH	F	SH	F
PMR-57 (S,T)	R	R	R	R	R	R	R	R	R	R
RHRC-50-1 (T,T)	R	R	R	R	R	R	R	R	R	R
PMR-69(S,S)	R	R	R	R	-	T	R	T	R	T
PMR-76 (S,S)	T	R	T	T	T	T	T	T	R	T
PMR-37 (S,S)	S	T	S	T	T	T	S	T	T	T
MI-2 (T,T)	T	R	T	R	T	R	T	R	T	R
EG-174(S,T)	S	R	S	R	S	R	S	R	S	R
AR-27 (S,T)	S	T	S	R	S	R	R	R	S	R
AR-28 (T,T)	S	R	-	R	R	R	-	R	-	R
AR-52(T,T)	R	R	R	R	R	R	R	R	T	R
D-76 (S,S)	R	R	T	R	T	R	T	R	T	R

R= Resistant, T= Tolerant, S=Susceptible; SH= Screen House, F=Field

Table 2: Reaction of Hybrids to Potato Virus Y (PVY) under Screen House and Field Condition in Chilli

	Perennial (R,R)		Punjab Lal (R,R)		Punjab Guchedhar (R,R)		Pant C-1 (R,R)		Tiwari (R,R)	
	SH	F	SH	F	SH	F	SH	F	SH	F
PMR-57 (T,R)	R	R	R	R	R	R	-	R	-	R
RHRC-50-1 (R,T)	R	R	-	R	R	R	-	R	-	R
PMR-69(T,R)	R	R	R	R	-	R	-	R	-	R
PMR-76 (R,R)	-	R	-	R	-	R	-	R	-	R
PMR-37 (R,R)	R	R	R	R	R	R	-	R	-	R
MI-2 (T,R)	R	R	T	R	R	R	R	R	R	R
EG-174(R,R)	R	R	R	R	R	R	S	R	R	R
AR-27 (R,R)	R	R	-	R	-	R	-	R	-	R
AR-28 (R,R)	T	R	-	R	R	R	R	R	T	R
AR-52(R,R)	-	R	-	R	-	R	-	R	T	R
D-76 (R,R)	T	R	-	R	-	R	-	R	-	R

R= Resistant, T= Tolerant, S=Susceptible; SH= Screen House, F=Field

Table 3: Reaction of Hybrids to Chilli Veinal Mottle Virus (CVMV) under Screen House and Field Conditi on in Chilli

	Perennial (R,R)		Punjab Lal (R,R)		Punjab Guchedhar (T,R)		Pant C-1 (R,R)		Tiwari (T,R)	
	SH	F	SH	F	SH	F	SH	F	SH	F
PMR-57 (T,R)	R	R	R	R	-	R	R	R	R	R
RHRC-50-1 (R,T)	R	R	R	R	R	R	R	R	R	R
PMR-69(T,S)	R	R	R	R	R	R	R	R	R	R
PMR-76 (T,T)	R	R	R	R	-	R	-	T	-	T
PMR-37 (R,T)	S	R	S	R	R	R	S	R	-	R
MI-2 (S,T)	S	R	S	R	S	R	S	R	S	R
EG-174(S,S)	T	R	S	R	S	R	S	R	S	R
AR-27 (S,S)	S	R	T	R	T	R	R	R	S	R
AR-28 (R,T)	S	R	-	R	R	R	T	R	T	R
AR-52(T,T)	-	R	R	R	-	R	-	R	-	R
D-76 (S,S)	-	R	-	R	-	R	-	R	-	R

R= Resistant, T= Tolerant, S=Susceptible; SH= Screen House, F=Field

Table 4: Reaction of Hybrids to Cucumber Mosaic Virus (CMV) under Screen House and Field Condition in Chilli

	Perennial (S,R)		Punjab Lal (T,R)		Punjab Guchedhar (S,T)		Pant C-1 (S,T)		Tiwari (S,S)	
	SH	F	SH	F	SH	F	SH	F	SH	F
PMR-57 (R,R)	R	R	-	R	R	R	-	T	R	R
RHRC-50-1 (R,R)	-	R	-	R	-	R	-	R	-	R
PMR-69(T,S)	R	R	-	R	R	R	R	T	R	R
PMR-76 (R,R)	-	R	-	T	-	T	R	R	-	T
PMR-37 (S,S)	S	R	R	R	-	R	-	R	-	R
MI-2 (S,S)	T	R	R	R	R	R	T	R	R	T
EG-174(S,S)	S	T	T	T	R	R	R	R	R	R
AR-27 (S,T)	T	R	-	R	-	R	-	R	-	R
AR-28 (T,T)	R	R	R	R	-	R	-	T	-	R
AR-52(T,T)	R	R	R	R	-	T	-	R	-	R
D-76 (S,S)	-	R	-	R	-	R	-	R	-	R

R= Resistant, T= Tolerant, S=Susceptible; SH= Screen House, F=Field